

University of Dundee

## Models of Ciliary Dysfunction

Shoemark, Amelia

*Published in:*  
American Journal of Respiratory Cell and Molecular Biology

*DOI:*  
[10.1165/rcmb.2018-0072ED](https://doi.org/10.1165/rcmb.2018-0072ED)

*Publication date:*  
2018

*Document Version*  
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

*Citation for published version (APA):*  
Shoemark, A. (2018). Models of Ciliary Dysfunction: Time to Expand. *American Journal of Respiratory Cell and Molecular Biology*, 59(3), 285-286. <https://doi.org/10.1165/rcmb.2018-0072ED>

### General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## **Editorial**

### **Models of ciliary dysfunction: time to expand**

Amelia Shoemark

Scottish Centre for Respiratory Research, University of Dundee, Dundee, UK

Royal Brompton Hospital, London, UK

Multiple motile cilia line our airways and beat in a co-ordinated pattern to transport mucus away from the lungs. This mucociliary escalator forms one of the first defences against foreign material entering the lower respiratory tract and mucociliary dysfunction leaves the respiratory tract vulnerable to infection. Ciliary dysfunction has been identified as a feature of several common airway diseases such as asthma, COPD and bronchiectasis, making improvement of ciliary function a tempting target for drug development (1, 2).

Primary ciliary dyskinesia (PCD) is an inherited respiratory condition affecting approximately 1:10,000 of the population (3). Failure of motile cilia to clear secretions from the ears, sinuses and lower airways results in otitis media, rhinitis, wet cough, recurrent upper and lower respiratory tract infections and bronchiectasis (3). Approximately 50% individuals with PCD have complete reversal of left-right symmetry of the internal organs (situs inversus), complex congenital heart disease or other laterality defects due to disrupted ciliary function in the embryonic node. Surprisingly, there are currently no licensed pharmaceutical products directed at correcting ciliary function. One of the obstacles for development of such therapies is the lack of laboratory models to reproducibly study therapeutic effect on a large scale.

Murine models of ciliary dysfunction have been developed but often, due to lack of functioning cilia in the brain, these murine models develop hydrocephalus and die (4, 5). Furthermore mice with mucociliary clearance dysfunction do not develop lung disease which mimics that of humans.

In-vitro air liquid interface (ALI) cultures of differentiated primary epithelial cells are useful models in the diagnosis of PCD (6). ALI cultures, usually grown from nasal brushings, have been shown to reproduce the primary ciliary phenotype in PCD whereas ciliary dyskinesia secondary to bacterial or viral infection normalises (7). However, use of primary ALI cultures at scale presents some difficulties. Firstly facilities are required for growth of human derived samples which are likely to be infected with pathogenic micro-organisms. The largest obstacle is that primary cells can't typically be expanded beyond 3-5 passages leaving limited numbers of cultures from a single donor. Consequently a large number of different volunteers with ciliary dysfunction willing to have a biopsy or a system to use donor lungs post mortem or post transplant is required to perform experiments at scale. Several methods have been developed to help overcome these difficulties by extending the differentiation potential of primary cells. These methods include, use of a murine fibroblast feeder layer, transformation of cells with BMI-1, and use of ROCK inhibitors to extend proliferation potential of basal cells while maintaining the potential to

differentiate into ciliated epithelial cells (8-10). A well described immortalised cell line which could be reproducibly differentiated at ALI into columnar epithelial cells with dyskinetic cilia would overcome many of these difficulties and allow studies to be scaled up. In the present issue of AJRCMB an article by researchers in Melbourne Australia describe a dyskinetic ciliary phenotype identified in an existing immortalised cell line (BCi-NS1) (11). When cultured at ALI ciliary beat frequency in these cells is lower than controls at ~4Hz and increases in response to known stimulus (ATP and IL-1 $\alpha$ ) demonstrating potential to test modifiers of ciliary beat frequency. The authors describe a circular ciliary beat pattern and central pair agenesis by transmission electron microscopy and propose that this model could be used for screening drugs to enhance ciliary beat frequency and pattern.

Interestingly in primary cells a circular beating of the cilia when visualised from above, is associated with a rare form of PCD accounting for approximately 10% patients with the condition (12). Circling cilia are associated with defects of the central pair projections or the proteins of the radial spoke head such as RSPH4a, RSPH1 and RSPH9 (13). It has been suggested that the electron microscopy phenotype results from a twisting of the central complex and eventual loss of the central pair as a result of the circular beat pattern (14). Cilia in the embryonic node which control right –left symmetry ordinarily lack a central pair of microtubules and beat with a circular beat pattern in health. Resultantly individuals with *RSPH1, 4a and 9* genes do not present with situs abnormalities, as nodal cilia are unaffected. A weakness of the study by Kuek et al, is the lack of known mechanism for the ciliary dyskinesia in the BCi-NS1 cell line. The authors have gone to some lengths to try and address this weakness, such as conducting immunofluorescent staining for RSPH1 proteins, and karyotyping with multicolour fluorescence in situ hybridisation for defects in chromosomal patterning. However no clear reason for the ciliary dyskinesia has been established and use of the model will likely be limited by this factor. Despite this, a ciliary dyskinesia cell line could form a useful resource to answer questions about ciliary beat frequency and pattern in rare and common diseases, as well as provide a reproducible model for manipulation of ciliary function. These cells along with alternatives created through emerging iPSc and CRISPR technology could be grown at ALI or as spheroids or organoids to allow an increase in the scale at which studies on ciliary dyskinesia can be reproducibly performed in the future.

1. Thomas B, Rutman A, Hirst RA, Haldar P, Wardlaw AJ, Bankart J, et al. Ciliary dysfunction and ultrastructural abnormalities are features of severe asthma. *J Allergy Clin Immunol.* 2010;126(4):722-9 e2.
2. de Jongh RU, Rutland J. Ciliary defects in healthy subjects, bronchiectasis, and primary ciliary dyskinesia. *Am J Respir Crit Care Med.* 1995;151(5):1559-67.
3. Lucas JS, Burgess A, Mitchison HM, Moya E, Williamson M, Hogg C. Diagnosis and management of primary ciliary dyskinesia. *Arch Dis Child.* 2014;99(9):850-6.
4. Ostrowski LE, Dutcher SK, Lo CW. Cilia and models for studying structure and function. *Proc Am Thorac Soc.* 2011;8(5):423-9.
5. Lucas JS, Adam EC, Goggin PM, Jackson CL, Powles-Glover N, Patel SH, et al. Static respiratory cilia associated with mutations in *Dnahc11/DNAH11*: a mouse model of PCD. *Hum Mutat.* 2012;33(3):495-503.
6. Lucas JS, Barbato A, Collins SA, Goutaki M, Behan L, Caudri D, et al. European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. *Eur Respir J.* 2017;49(1).

7. Hirst RA, Jackson CL, Coles JL, Williams G, Rutman A, Goggin PM, et al. Culture of primary ciliary dyskinesia epithelial cells at air-liquid interface can alter ciliary phenotype but remains a robust and informative diagnostic aid. *PLoS One*. 2014;9(2):e89675.
8. Munye MM, Shoemark A, Hirst RA, Delhove JM, Sharp TV, McKay TR, et al. BMI-1 extends proliferative potential of human bronchial epithelial cells while retaining their mucociliary differentiation capacity. *Am J Physiol Lung Cell Mol Physiol*. 2017;312(2):L258-L67.
9. Butler CR, Hynds RE, Gowers KH, Lee Ddo H, Brown JM, Crowley C, et al. Rapid Expansion of Human Epithelial Stem Cells Suitable for Airway Tissue Engineering. *Am J Respir Crit Care Med*. 2016;194(2):156-68.
10. Mou H, Vinarsky V, Tata PR, Brazauskas K, Choi SH, Crooke AK, et al. Dual SMAD Signaling Inhibition Enables Long-Term Expansion of Diverse Epithelial Basal Cells. *Cell Stem Cell*. 2016;19(2):217-31.
11. Kuek LE, Griffin P, Martinello P, Graham AN, Kalitsis P, Robinson PJ, et al. Identification of an Immortalised Human Airway Epithelial Cell Line with Dyskinetic Cilia. *Am J Respir Cell Mol Biol*. 2018.
12. Chilvers MA, Rutman A, O'Callaghan C. Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia. *J Allergy Clin Immunol*. 2003;112(3):518-24.
13. Castleman VH, Romio L, Chodhari R, Hirst RA, de Castro SC, Parker KA, et al. Mutations in radial spoke head protein genes RSPH9 and RSPH4A cause primary ciliary dyskinesia with central-microtubular-pair abnormalities. *Am J Hum Genet*. 2009;84(2):197-209.
14. Burgoyne T, Lewis A, Dewar A, Luther P, Hogg C, Shoemark A, et al. Characterizing the ultrastructure of primary ciliary dyskinesia transposition defect using electron tomography. *Cytoskeleton (Hoboken)*. 2014;71(5):294-301.